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Pretreatment and Substrate Evaluation for the Enzymatic Hydrolysis of Cellulosic Wastes

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PRETREATMENTS AND SUBSTRATE EVALUATION FOR THE ENZYMATIC HYDROLYSIS OF CELLULOSIC WASTES

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by

Leo A. Spano
Thomas H. Tassinari
Charles F. Macy
Edward D. Black

Pollution Abatement Division
Food Sciences Laboratory
U.S. Army Natick Research and Development Command
Natick, Massachusetts 01760

Contract No. EPA-IAG-05-0758

Project Officer

Charles J. Rogers
Solid and Hazardous Waste Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

The objective of this study was to determine the applicability of various pretreatment methods for conversion of cellulose to glucose and other sugars. A combination of pretreatments, two-roll milling followed by liquid ammonia, affected significant increases in the enzymatic hydrolysis yield of glucose from wood shavings and hammer milled newspaper.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

Presented are initial studies aimed at determining the applicability of various pretreatment methods and existing cellulosic wastes to a process for the conversion of cellulose to glucose and other reducing sugars. In this process, a cellulase from a mutant of Trichoderma viride is used.

An extensive literature survey of cellulose pretreatment methods was conducted followed by experimental work consisting of an evaluation of pulp and paper mill primary sludge waste and investigation of four pretreatments, viz., modified hydropulping, two-roll milling, attritor milling and anhydrous liquid ammonia. Reference point for the sugar yield obtained for a particular substrate is taken as the value for ball milled newspaper (BMNP) hydrolyzed at the same time. Some primary sludge discharges from pulp and paper mills, without further pretreatment, exhibit hydrolysis yields higher than BMNP. Hydrolysis of hammer milled newspaper that had been treated in a type of hydropulping mill resulted in saccharification yields that were approximately 80% of those of BMNP. Preliminary results with an attritor mill pretreatment of newspaper are sufficiently promising to warrant a more extended investigation.

Pretreatment using differential speed two-roll mills has significantly enhanced the susceptibility of diverse cellulosic substrates such as newspaper, cotton, pine and maple. With processing times of less than ten minutes on a six-inch (roll diameter) mill, hydrolysis yield improvements were two and twelve-fold for newspaper and cotton respectively. Power requirements for processing waste newspaper on a three-inch laboratory mill are estimated to be 20% less than for a commercial ball mill. Anhydrous liquid ammonia treatments of hardwood birch and maple sawdusts rendered them more susceptible to enzymatic hydrolysis than substrates from a softwood source, e.g., white pine and newspaper. A combination of pretreatments, two-roll milling followed by liquid ammonia, affected significant increases in hydrolysis yield for maplewood shavings and hammer milled newspaper.

This report is submitted in fulfillment of contract No. EPA-IAG-05-0758 by U.S. Army Natick Research and Development Command under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period 16 July 1975 to 15 July 1976 and work was completed as of 4 August 1976.

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SECTION I

INTRODUCTION

Cellulose, the world's most abundant organic compound, is a replenishable resource generated at an estimated annual rate of 100 billion tons. This is equivalent to 150 lbs/day for every man, woman, and child in the world.

Ultimately, most of the cellulose utilized by man becomes part of the solid waste stream. In the United States, significant amounts of cellulosic materials are found in solid waste derived from industrial, agricultural and municipal sources. Disposal of these cellulosic wastes in an environmentally acceptable manner is a problem which must be dealt with. Landfilling will only be practical in the short term as land for this purpose becomes scarce. An inexpensive, non-polluting incinerator technology has yet to be developed. The Natick Research and Development Command (NARADCOM) Process for the Conversion of Cellulose to Glucose offers a potential solution to this waste disposal problem.

In the process, a mutant of Trichoderma viride is grown on a cellulose substrate, and the extracellular enzyme complex produced is separated from the mycelia. Waste cellulose is then hydrolyzed at 50°C, pH 4.8 in the presence of the fungus derived cellulase yielding a syrup consisting primarily of glucose with lesser amounts of cellobiose and xylose.

To minimize hydrolysis time on the way toward making process economics acceptable for commercial application, it is necessary for most cellulosic wastes to be pretreated. In the past, mechanical and chemical pretreatments have been tried with improvements noted. However, none have been economically successful.

NARADCOM, supported by the Environmental Protection Agency Agreement No. EPA-IAG-D7-0758, has continued pretreatment studies initiated at Natick. The results contained herein are in fulfillment of this Agreement and stem from the following objectives: (a) to search for pretreatment methods which will enhance susceptibility of cellulosic wastes to enzymatic hydrolysis by T. viride cellulase while minimizing costs, and (b) evaluate cellulosic wastes with respect to their potential as acceptable substrates for the Cellulose Conversion Process.

This report consists of a review of pertinent background information from the literature and the results and discussion of experiments to date.

SECTION 2

CONCLUSIONS

The results of an extensive literature survey indicate that important factors in increasing susceptibility of cellulosics to enzymatic hydrolysis are the extent and type of crystallinity and lignin content of the substrate.

Most pulp and paper mill primary sludges from chemical pulping operations could be used with no pretreatment to enhance susceptibility to enzymatic hydrolysis by T. viride cellulase.

The Retting Machine, a modified hydropulper, provided only modest improvements in susceptibility of newspaper to enzymatic hydrolysis with low power demands and under a variety of operating conditions. Hence, further experimental studies of this machine were considered not desirable.

Preliminary results indicate that attritor milling significantly increases susceptibility of newspaper to enzymatic hydrolysis by T. viride cellulase.

Two-roll milling of newspaper, cotton, and wood chips has been shown effective in enhancing susceptibility to enzymatic hydrolysis. This was accomplished using 3, 6, and 10-inch diameter mills.

Power requirements for the three-inch two-roll laboratory mill are at least 20% less than for a commercially ball milled sample and are expected to decrease as scale-up to larger mills proceeds.

The two most important factors discovered to date in determining the effectiveness of two-roll mill processing are roll clearance and processing time. Data collected thus far indicate that large-scale mills using short processing times, and high loadings may be possible.

Laboratory two-roll milled newspaper has a low sedimentation volume (lower than commercially ball milled newspaper) thus making it conceivable to slurry at high concentrations in the hydrolysis vessel of a production scale process.

Studies of anhydrous liquid ammonia treatment of cellulosic materials provide the potential of a chemical process in which the pretreating agent can be recycled.

Materials originating from hardwood sources appear more amenable to ammonia treatment than those from softwood sources.

The possibility of a combined mechanical-chemical pretreatment is indicated by experiments on two-roll milling followed by treatment with anhydrous liquid ammonia.

While laboratory data indicate significant advances in the pretreatment of waste cellulosic materials, considerable further effort is necessary to develop commercially practical methods which will contribute substantially to the economic viability of the enzymatic conversion process. Optimum equipment design, energy requirements, processing techniques and other system variables will require extensive investigation.

SECTION 3

RECOMMENDATIONS

Continue scale-up of two-roll milling, paying particular attention to power requirements and capacities as well as effects of moisture, temperature, roll speed ratio, and roll surface.

Continue further liquid ammonia pretreatment studies concentrating on effects due to substrate composition and optimizing operating conditions.

Investigate other gas phase treatments such as SO_2 which might be more effective in treating unresponsive (to liquid ammonia) substrates such as softwood.

Expand studies of combination chemical/two-roll milling treatments.

Initiate studies of the effectiveness and economics of attritor milling.

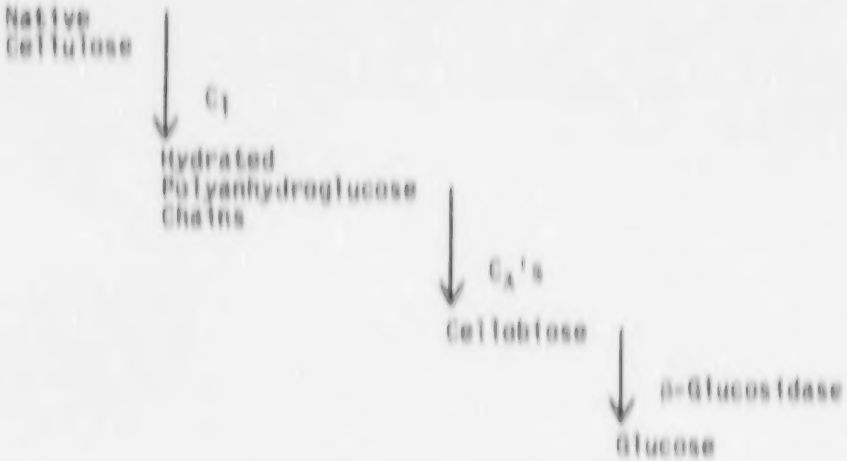
Study the nature of chemical and/or physical changes occurring in cellulosic materials as a result of pretreatment. Important factors to be considered include changes in crystallinity, degree of polymerization and the lignin-carbohydrate complex.

SECTION 4

BACKGROUND REVIEW

PROPERTIES OF CELLULOSE AFFECTED BY PRETREATMENT

The action of the cellulase enzyme complex (C_1, C_x) on cellulose can be approximated by the following reaction scheme (1).



In actuality, the reactions are quite complex with multi-component enzymes and many reaction pathways to be considered (2, 3).

Susceptibility of cellulose and cellulosic wastes to enzymatic hydrolysis is greatly affected by a number of features of the cellulose-enzyme system. These have been discussed by Cowling and Brown (4) and are summarized as follows:

1. moisture content
2. size and diffusibility of the enzyme molecules in relation to the capillary structure of cellulose

3. degree of crystallinity of the cellulose

4. unit cell dimensions of the crystallites present

5. conformation and steric rigidity of the anhydroglucose units

6. degree of polymerization of the cellulose

7. nature of the substances with which the cellulase is associated

8. nature and concentration and distribution of substituent groups

To a greater or lesser degree it is possible to alter these factors by physical and/or chemical means prior to hydrolysis in order to facilitate the action of the enzyme. It is evident that such pretreatment can have an important bearing on the overall costs of an effective utilization of waste cellulose through an enzymatic hydrolysis process.

Three aspects of cellulosic materials are considered to be particularly significant with respect to susceptibility to enzymatic hydrolysis and as potential bases of effective pretreatment. Highly crystalline, i.e., ordered, regions of a substrate are more resistant than the less ordered amorphous regions. Secondly, the presence of lignin and its manner of association with the cellulose can interfere with the hydrolysis. Reduction in the degree of polymerization of cellulose leads to an enhancement of the enzymatic hydrolysis. The following summary indicates previous work that has been directed to these subjects.

Crystallinity

By x-ray diffraction analysis, cellulose has four known stable crystal lattices (5). Cellulose I, the native form, is found in cotton, ramie, wood, jute, etc., while cellulose II is formed by treating cellulose I with alkali solutions and is found in viscose process products (i.e., rayon, cellophane).

Cellulose III is formed by treating cellulose I or II with liquid ammonia (6) or by decomposition of the ethylamine-cellulose complex (7). Cellulose IV is prepared by heating cellulose III in glycerin at 260°C (8).

Ball milling has been demonstrated to induce crystalline modifications in α-cellulose pulps (9, 10). Dry ball milling results in complete destruction of the crystalline lattice as determined by x-ray diffraction. However, upon wetting and redrying, reversion to the native cellulose I form occurs in cases where the ball milling time was insufficient. In the Caulfield and Stuffes report (11) ball milling times of six hours gave a product whose crystalline structure was entirely cellulose II after being soaked in water at 40°C and dried. The extent of crystallinity as measured by the crystalline index was roughly one-half of the original non-milled cellulose pulp.

After three hours of ball milling, the wetted and dried product's crystalline phase was a mixture of cellulose I and cellulose II.

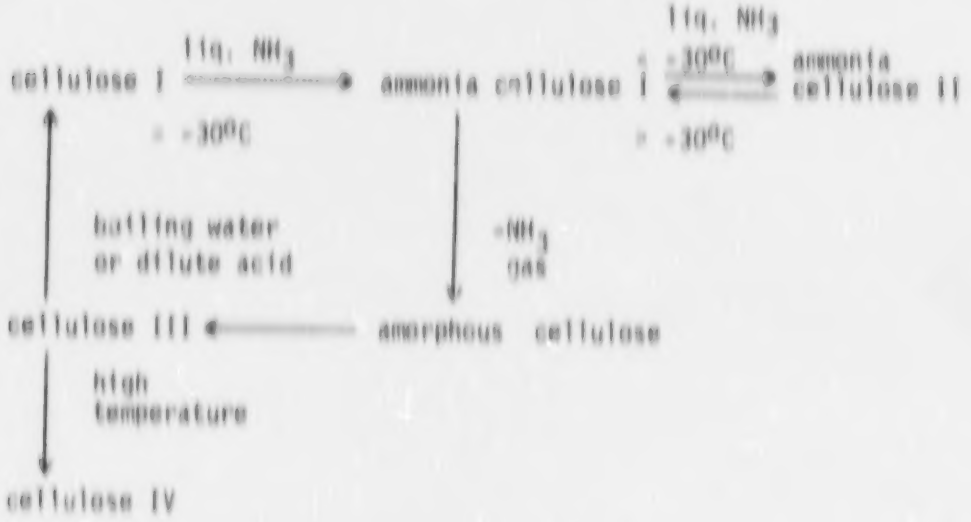
It has been proposed that the mercerization process causes a sequence of metastable crystalline structural changes leading to cellulose II (12). The native cellulose I form of ramie fibers when treated with caustic soda at room temperature is transformed into soda cellulose. Upon alkali removal by washing with water at room temperature, the soda cellulose decomposes to give water cellulose. As the fibers are dried, the water cellulose changes to cellulose II.

Clark and Terford (13) have determined by x-ray diffraction the % crystallinity of 23 commercial paper pulps. The groundwood pulps had higher amorphous contents (about 44%) than the chemical pulps (42-39% amorphous). This is attributed to the use of mechanical equipment (stone grinders, refiners) in groundwood production and the resulting breakdown of crystalline regions during fiber separation. Bleaching was demonstrated to increase slightly the percent crystallinity of both chemical and groundwood pulps.

Measuring x-ray diffraction peak widths, Parks (14) estimated the size of ordered regions of a variety of sulfite and kraft pulps. The sulfites contained small ordered regions which are uniform in perfection of order, whereas the kraft pulps have intermediate size ordered regions with varying degrees of perfection.

Anhydrous liquid ammonia affects cellulose and cellulosic materials in a number of ways.

Of particular interest are the swelling and transitions in crystalline forms, as determined by x-ray diffraction measurements, that can be brought about through treatment with anhydrous ammonia (6, 15, 16). Schuerch (17) summarizes the crystal lattice changes schematically as follows:



Lewin and Roldan (18) present evidence for a disordered cellulose and an interpretation that the amorphous cellulose of Schuerch (17) consists of a mixture of less ordered cellulose I and cellulose III. The ammonia-cellulose system is sensitive to temperature and pressure and to the manner in which ammonia is removed. In general, evaporation of the ammonia leads to retention of the less ordered structure and the presence of water or dilute acid in the removal process tends towards reconversion to cellulose I.

The occurrence of small amounts of ammonium salts and amides in the residues of woods treated with liquid ammonia indicate reaction with uronic acids and uronic acid ester links present in the original wood (19).

An increase of accessibility of different cellulose structures pretreated with liquid ammonia was observed by Schleicher et al. (20). Considerable increases in the reaction rate and degree of conversion were found for the reactions xanthogenation, carboxymethylation, and cyanoethylation.

The phenomenon of the plasticization of wood, similar to plasticizing with steam, has been described by Schuerch (17, 21). In many instances, the shaping of wood may be carried out quite easily and in shorter times with liquid ammonia than with steam. This is attributable to the ability of the ammonia not only to solvate the amorphous regions of the cellulose but also to enlarge and relax the crystal lattice.

Millett et al., (33) review the use of ammonia, aqueous, gaseous or liquid in attempts to upgrade the feeding value of lignocellulosic materials.

In general, strong interactions of an acid-base nature between ammonia and the hydroxyl groups and hydrogen bonds of cellulose are to be expected.

Disruption of the Lignin Carbohydrate Complex

The nature of the association between lignin and carbohydrate in wood has been debated for many years. Three main theories are prevalent: (a) hydrogen bonding between constituents, (b) covalent chemical bonds, and (c) incrustation where the three dimensional lignin network encases the cellulose, thereby preventing easy access of enzyme molecules.

Brownell and West (22) have presented some evidence for the presence of a lignin-carbohydrate chemical bond in wood. Through fractionation of ball milled wood which has been solubilized with ethylene oxide, they were able to obtain one-half of the carbohydrate essentially lignin-free while three-quarters of the initial lignin present was found in fractions containing carbohydrate. They discount the possibility of incrustation since the wood was entirely solubilized.

On the other hand, Pew and Weyna (23) have been advocates of the incrustation theory. They contend that evidence presented for the existance of covalent bonds is incomplete and that chemical bonds alone could not explain the total resistance of wood to cellulolytic enzymes. It is their contention that even ball milled wood particles as small as several microns con-

stitute a "cage" of lignin in which carbohydrate molecules are enmeshed. The exposed carbohydrate would allow ready dispersal in cellulose solvents and in lignin dissolving solvents which have the additional tendency to dissolve amorphous cellulose. A model experiment in which holocellulose was impregnated and cured with phenolic resin tended to support their view.

With respect to hydrogen bonding, it was concluded based on their work and that of Lindberg (24) that the strength of hydrogen bonding of lignin to carbohydrate would be weak due to the highly amorphous character of lignin.

Consider now some of the more important treatments believed to disrupt the ligno-carbohydrate complex, however it may be constituted.

Gratzen et al., (29) have shown that 295 kHz ultrasonic vibrations break the cellulose-lignin bond of wood (1% aqueous slurry) so that cellulose is soluble in cuprammonium solvent.

Millett and associates (33) have been able to enhance significantly the enzymatic saccharification of hardwoods and softwoods using a moist SO_2 treatment. Although no lignin was removed during the processing, Klason values for the hardwoods were less than 9% compared to the initial values of greater than 20%. Similar less dramatic results were seen for the softwoods. Apparently some depolymerization of the lignin occurred disrupting the lignin-carbohydrate complex.

Yan and Purves (26, 27) examined delignification of a number of substrates via liquid ammonia. Employing temperatures and pressures up to 100°C and 900 p.s.i., only small amounts of material were extracted from the softwood spruce but 25 to 30% of the lignin was removed from the hardwoods beech, birch, and maple, and 52% from rye straw.

In the $Fe^{++} + H_2O_2$ pretreatment of sweetgum wood, Koenigs (28) has, without lignin removal, determined that Klason lignin of the untreated wood was 18.1% as opposed to 8.4% for the treated sweetgum thus indicating an alteration of the lignin network.

For a brief review of significant commercial and experimental lignin removal methods, see Appendix A.

Reduction of the Degree of Polymerization

It is well documented in the literature (10, 11, 29) that ball milling pure cellulose results in substantial decreases in degree of polymerization (DP). The fine structure of the cellulose to be milled apparently determines in large measure the extent of degradation to be expected for a given length of time in the mill. For example, Leopold and Moulik (30) using a vibratory ball mill, found that kraft pulp is depolymerized more than twice as rapidly as sulfite pulps. They attributed this behavior to the presence of smaller and more numerous crystallites in the kraft pulp thus making it less able to

delocalize mechanical energy resulting in destruction of ordered regions as well as cleavage of covalent bonds.

Leopold and Moulik also observed that 10 hours of dry milling is roughly equivalent to 50 hours of milling in water (liquid to solid ratio = 200/1) for lignin containing kraft and sulfite pulps. Kraft pulp degraded almost four times as fast as sulfite pulp on wet milling which they contend is due to the greater ability of kraft pulp to be plasticized by water.

Howsmon and Marchessault (10) noted that rotary ball milling acetate-grade wood pulp in dry air resulted in greater reduction in degree of polymerization than a carbon dioxide atmosphere. They theorize that the presence of oxygen increases the extent of chain cleavage probably by acting as a radical acceptor of the free radical chains produced by mechanical action. The peroxy radical probably then initiates further chain scission.

Morehead (31) showed that ultrasonic vibration reduces the DP values of cellulose but not below the limiting values obtained by acid hydrolysis.

Cotton linters and alpha sulfite pulp were depolymerized extensively by high voltage cathode rays at dosage levels above 5×10^5 equivalent roentgens (32). They depolymerized at practically the same rate resulting in products with nearly average chain length. At a dose of 6×10^6 equivalent roentgens, the cotton linters were converted to water soluble materials.

CHEMICAL PRETREATMENTS

Chemical pretreatments of cellulosic materials are of potential benefit in the preparation of substrates for enzymatic hydrolysis. Factors that may be modified in order to improve susceptibility to hydrolysis include swelling, degree of polymerization, hydrogen bonding, relative extent of crystalline and amorphous regions, amount of lignin present and the nature of its association with cellulose. Disadvantages of chemical pretreatment are the possible occurrence of undesirable side reactions, as for example, derivative formation and oxidation. Also, the chemical media themselves may present problems in their disposal without contributing further to the contamination of the environment.

Millett et al., (33) reported that mercerization of cotton and ramie yielded 40-50% increases in acid hydrolysis rates using constant boiling hydrochloric acid. However, mercerized hemlock pulps showed no improvement. They also investigated dilute alkali pretreatment (5-10% NaOH) of a variety of wood species and measured in vitro digestibility by determining substrate weight loss following incubation with rumen fluid under prescribed conditions (34). Softwoods (26-35% lignin), in general, were more resistant to alkali treatment than hardwoods (17-28% lignin), indicative of the effect of lignin content.

Baker (35) has produced a series of hard and softwood kraft pulps of varying residual lignin contents and measured in vitro digestibility. The extent of delignification required to achieve 60% digestibility (equivalent to a good quality hay), for Douglas fir softwood was 73% whi: only 25% for white birch hardwood. Once again the relationship of lignin content to digestibility was demonstrated.

Mandels, Monte, and Nystrom (36) treated hammer milled newspaper with 2% NaOH at 70°C for 90 minutes and noted moderate improvement with respect to susceptibility to enzymatic hydrolysis by T. viride cellulase. If the treated sample was dried, susceptibility was markedly reduced and in fact, less than the untreated sample, perhaps indicative of crystalline changes within the cellulose microstructure.

More impressive results were noted for viscose and cuprammonium regenerated newspapers after a 48-hour saccharification. The cuprammonium treatment provided a greater than three-fold improvement in susceptibility while the viscose newspaper was 2.5 times better than untreated newspaper. Drying these samples and retesting for susceptibility, an initial lag was observed but disappeared by 48 hours as the undried and dried samples were equal.

Andren et al., (37) tested commercial sulfite and kraft process pulps for susceptibility to T. viride cellulase hydrolysis. Both were considered excellent substrates with the sulfite completely saccharified at 48 hours. Bemenberg rayon (wet) was also evaluated and found to be excellent with a 72% saccharification at 48 hours.

Using a T. viride derived cellulase, Toyama and Ogawa (38) examined the effects on decomposition (weight loss) of the alkaline and peracetic acid pretreatment of rice straw, bagasse and two wood species. Partial delignification of bagasse and rice straw specimens by boiling in 1% NaOH for 3 hours or alternatively 1 hour at 120°C, showed 55% and 48% weight losses at 48 hours respectively as a result of enzymatic hydrolysis. Boiling in 20% peracetic acid for 1 hour followed by autoclaving at 120°C in 1% NaOH resulted in decompositions of 94.1% for the hardwood (Machilus thunbergii) and 7:.9% for the softwood (Cryptomeria japonica) at 48 hours. Residual lignin contents were 18% for the softwood (originally 32%) and 15% for the hardwood, down from 30%. Applying the NaOH treatment first followed by the peracetic acid was not effective in enhancing 48-hour decomposition of the softwood (only 22% decomposition), even though there was essentially no difference for the hardwood decomposition.

Koenigs (39) using sweetgum woodmeal slurried with 0.44 mM Fe^{++} and 1% H_2O_2 controlled at room temperature and pH 4.2 for three days, showed a 50-55% weight loss after 64-hour treatment with Onazuka cellulase derived from T. viride. Significant reduction in degree of polymerization and enhancement of alkali solubility were noted for the treated material. In addition, hemicellulose loss was high and apparently lignin structure was altered. It was proposed that this Fe^{++} catalyzed system may be acting similar to brown rot fungi, producers of extracellular H_2O_2.

The action of the $Fe^{++} + H_2O_2$ system on cattle feed lot waste and its effect on hydrolysis by T. viride cellulase were examined by Elmund, Grant and Morrison (40). Feed lot waste slurried at 5% was reacted with 150 mM H_2O_2 and 10 mM ferrous sulfate at 22°C for 6-8 hours. Results from a 24-hour hydrolysis showed 46% of the cellulose in feed lot waste was converted to reducing sugar.

Ballamy (41) investigated the 48-hour growth at 55°C of Thermoactinomyces on pretreated feed lot waste fiber. With no pretreatment, only 15-20% of the cellulose was utilized. Sodium hydroxide treatment (0.05 M, 23°C, 4 hours), resulted in 74-81% cellulose utilization while treating with anhydrous liquid ammonia for 10 hours at 23°C and 10 atmospheres netted a 72-80% cellulose utilization.

Aqueous ammonia treatment has been demonstrated by Han and Callihan (42) to increase the rice straw utilization by Cellulumonas bacteria from 29% to 57%.

Liquid or gaseous anhydrous ammonia, another strong swelling agent, has been used on three wood species and evaluated using a 5-day incubation period in vitro assay (43). Aspen sawdust treated with liquid ammonia for 1 hour attained 50% digestibility coefficient whereas Sitka spruce and red oak digestibilities were only 25 and 10% respectively.

Disrupting the lignin-carbohydrate complex without significant removal of either component can be accomplished with sulfur dioxide. Operating with moist sawdust (water/wood = 3/1) and gaseous SO_2 under pressure for 2-3 hours at 210°C, Millett et al., (33) have obtained impressive results on both hardwoods and softwoods with Onazuka cellulase (derived from T. viride). Almost quantitative conversions of carbohydrate to sugars were attained after 48-hour saccharification of the hardwoods. Meanwhile, the two softwoods were 70-85% converted.

MECHANICAL PRETREATMENTS

The mechanical pretreatment area has received little attention as is evident from the relatively few publications found in the literature. Pew and Weyna (23) measured the effects of vibratory ball milling on the digestion (4-6 days) of spruce and aspen sawdusts using T. viride cellulase in 5% slurries at pH 4.6 and 40°C. As grinding time increased, solubilization of carbohydrate by enzyme increased to a 96% level for both woods after 8 hours (spruce) and 5 hours (aspen) of ball milling.

Mandels et al., (36) investigated hammer milling, ball milling, fluid energy milling and colloid milling, finding ball milling best due to its capacity to size reduce effectively while providing high bulk density and maximum susceptibility. Among the waste substrates treated were newspaper, bagasse, rice hulls, rumen fibers, corrugated fiberboard, and computer cards. Following are significant results of the Mandels' study with respect to

susceptibility of substrates to enzymatic hydrolysis by T. viride cellulase:

Hammer Milling

This is an impact mill consisting of a rotor to which a set of hammers are attached. As the rotor turns rapidly, the hammers impact the material against a breaker plate exerting compressive, shear, and tensive forces. The process is repeated until the material is small enough to pass through a screen at the bottom of the machine.

Results with newspaper showed virtually no improvement in susceptibility using a variety of screen sizes. In fact, for the finer screen sizes, susceptibility was less than for the newspaper feed material.

Fluid Energy Milling

Particles to be size reduced are entrained in high velocity gas or vapor streams and impacted against a target plate or opposing entrained particles. Product is removed using an internal air classifier.

Feeding between 0.036 kg/hr and 2.6 kg/hr and using both the target plate/nozzle and double opposed nozzle combinations, only modest susceptibility improvements were noted over the untreated newspaper. For this reason, plus the high operating costs and low production rates, this type of milling was considered unsatisfactory.

Colloid Milling

This attriting machine consists of two disks revolving in opposite directions at peripheral speeds between 10 and 50 m/sec and set close to each other. Feed in the form of a slurry is passed between the disks where particles impact each other and in the process are reduced in size and dispersed.

Operating at gap settings between 0.001 and 0.005 inches and with 2% water slurries of newspaper, only modest improvements in susceptibilities were observed. This fact along with high operating costs, led the authors to conclude that colloid milling would not be a satisfactory method of substrate preparation.

Ball Milling

The distinctive feature of a ball mill is the use of balls (steel, ceramic, etc.), filling about one-half the volume of a rotating shell. The feed material is placed within the shell, impacts the balls as tumbling occurs and in so doing is size reduced.

Ball milling was the most effective pretreatment found in the Mandels' study. Newspaper after 1 day of milling in a pot mill was 46.8% saccharified at 24 hours as compared to the hammer milled newspaper control which was only 23% saccharified. The major drawback to ball milling pretreatment is high power consumption making operating costs excessive.

IRRADIATION

The basic effects of ionizing radiation on cellulose have been reviewed by Arthur (44). In general, radiation initiates an oxidative degradation of the cellulose molecule. Dehydrogenation, destruction of anhydroglucose units to yield carbon monoxide and carbon dioxide and cellulosic chain cleavage to yield an homologous series of products are observed. Oxidative products include carboxyl and carbonyl compounds.

High voltage cathode ray electrons were used by Saeman et al., (45) to treat alpha cellulose wood pulp and cotton linters. Extent of dilute acid hydrolysis (0.1 N H_2SO_4 at 180°C) was used to determine treatment effectiveness. An optimum dosage of 10^5 equivalent roentgens resulted in overall sugar yields (carbohydrate decomposition included) of 70.2%, and 62.1% for the wood pulp and cotton linters respectively. Untreated wood pulp and cotton linters gave respective overall sugar yields of only 33% and 23%.

A combination thermal and linear accelerator high-energy electron beam radiation treatment was investigated by Millett and Goedken (32) using cotton linters and a nitration grade softwood sulfite pulp. Sugar production via dilute acid hydrolysis (0.1 N H_2SO_4 at 180°C) was measured to determine effectiveness of treatment. A dosage of 10^5 equivalent roentgens, the level at which irradiation begins to significantly affect cellulose structure, was applied since it is also the maximum permissible dosage from an economic standpoint. Best results were obtained by irradiating first followed by heat treatment at 200°C for periods up to 16 hours. Maximum sugar yield increased from 22.5% to 28.5%. Irradiation alone at 10^5 equivalent roentgens improved sugar yield from 22.5% to only 24.5%.

Even though the hydrolytic activity of cellulose was enhanced by this dual pretreatment, the authors concluded that the improvement was not sufficient to be of potential commercial significance.

Kelly (46) studied the effects of gamma radiation on the dilute-acid hydrolysis of cellulose. He found an enhancement of glucose yields due to the radiation that was too low to be industrially attractive. Imamura et al., comparing ^{60}Co gamma-ray and electron beam irradiation found little difference in the extent of depolymerization of cellulose by the two types of radiation (47).

SECTION 5

EXPERIMENTAL RESULTS AND DISCUSSION

EVALUATION OF PRIMARY SLUDGES

The spent process waters discharged from pulp and paper manufacture contain, among other constituents, suspended solids some of which are biodegradable. These solids contain, depending upon the source, varying amounts of non-usable short fibers, lignin, tannins, and various inorganics such as dirt, pigments, and fillers. Through sedimentation, filtration and/or flotation, suspended solids are separated from the effluent waste stream and the resulting residue (primary sludge) dewatered for subsequent landfill disposal or burning.

In Table 1 data compiled from Genn et al., (48) is presented on the expected levels of suspended solids in effluents from various pulp and paper mill processes, and their ash contents. Note that in special cases such as fine paper products, it is possible to find as much as 50% ash. However, in most processes, ash content is less than 15%. Also presented in this table are values representing the effectiveness of total suspended solids removal by clarifiers for selected mill processes. Except for deinking operations, a significant portion of the total suspended solids (80-90%) is being effectively removed from waste streams and hence the cellulosic fractions could be made available for subsequent utilization such as enzymatic conversion to sugars.

Dr. Edward Soltes (St. Regis Paper Co., West Nyack, NY) has provided estimates of wood pulp production in the major pulp and paper manufacturing states of this country (Table 2). Assuming that primary sludges account for 1% of the 81,500 ton/day total production, then an estimated 2445 tons/day (dry basis) are available. In addition, about 75% are derived from chemical operations (i.e., kraft and sulfite).

Susceptibility data of nine primary sludges submitted by major Northwest based paper companies is presented in Table 3. These sludges were evaluated as received (generally 50 to 80% moisture content) according to the incubation and DNS reducing sugar determination procedures described in Appendix B.

Relative yield is defined as the ratio of total reducing sugar concentration from the hydrolyzed substrate in question to that of ball milled newspaper. A value greater than or equal to 1.0 is considered good whereas between 0.69 and 0.99 fair. Less than 0.69 (hammer milled newspaper) the

Table 1. Pulp and Paper Mill Effluent Suspended Solids, Ash Content and Total Suspended Solids Reduction Effected by Clarifier

Product	Suspended Solids (lb/ton)	% Ash	% Reduction in Suspended Solids
Tissues	10-50	5-15	91
Fiber and Felts	60-150	10-25	--
Deinking	200-500	25-50	60
Newsprint	20-40	less than 10	92
Fine Paper	75-100	10-50	87
Bleached Kraft	5-10	less than 10	81
Semi-chemical Pulp	50-100	less than 10	--
Sulphite Pulp	35-50	less than 10	--

Table 2. Estimated Wood Pulp Production (tons/day) in the Major Pulp and Paper Manufacturing Areas of the U.S. (1975)

Location	Kraft	Sulfite	Groundwood	Semi-chemical	Total
Alabama	10,345	--	500	725	11,570
Florida	9,095	--	--	200	9,295
Georgia	12,470	--	300	640	13,410
Louisiana	9,560	--	570	1,160	11,290
Maine	2,705	1,445	3,138	--	7,368
Mississippi	4,615	--	250	--	4,865
Oregon	5,215	790	1,390	625	8,020
Washington	5,460	3,992	739	825	11,016
Wisconsin	1,284	1,462	900	1,020	4,666
Totals	60,829	7,689	7,787	5,195	81,500

Table 3. Reducing Sugar Yields of Various Pulp and Paper Mill Sludges Relative to Ball Milled Newspaper

Primary Sludge	Processing Source	Rating	Relative Yield 1 hr	5 hr	12 hr	24 hr
1	100% sulfite pulp	good	1.98	1.18	1.26	1.44
2	90% kraft 10% sulfite 10% bleached	good	1.20	1.21	1.23	1.28
3	100% kraft	good	1.16	1.08	1.09	1.24
4	80% kraft 20% groundwood 100% bleached	good	1.06	1.13	1.09	1.19
5	100% sulfite 75% bleached	good	1.13	1.03	1.05	1.10
6	100% kraft 5-10% bleached	fair	0.88	0.83	0.87	0.96
7	40% sulfite 60% refiner groundwood	fair	1.00	0.82	0.84	0.85
8	33% sulfite 67% refiner groundwood	fair	0.87	0.73	0.71	0.71
9	hammer milled newspaper control		0.77	0.73	0.66	0.62
10	40% groundwood 5% sulfite 45% bark & wood-log debris	poor	0.66	0.57	0.51	0.52

substrate is deemed poor. This rating system is based upon economic estimates for enzymatic process viability.

Most chemically derived sludges were good substrates and could be used without pretreatment to enhance susceptibility to enzymatic hydrolysis. As the content of groundwood increased in the sludges, the susceptibility decreased and approached hammer milled newspaper which is mostly groundwood. Even less susceptible was sludge number 10 containing 45% bark and wood-log debris.

Based on evidence found in the literature, the results obtained here are reasonable. Susceptibility improvements for groundwood over native wood

would be expected due to decreased crystalline content as a result of mechanical processing. The good results for sulfite and kraft derived sludges correlate well with data previously reported for sulfite and kraft pulps. Reactivity enhancements for sludges with high chemical fiber contents over high groundwood fiber sludges are probably due to differences in lignin content and the extent and types of molecular order in the cellulosic fraction of each sludge.

MODIFIED HYDROPULPING

Retting Industries, Inc. under Contract DAAG-17-76-C-0014 attempted to produce a better and less expensive method of pretreating cellulosic materials to make them more effective as a substrate in the enzymatic hydrolysis of cellulose to soluble sugars. This study was confined to waste newspaper.

The Retting mill is a simplified version of the "Pulping Machine" described in S. G. Stapley et al., expired U.S. patent no. 2,525,772 dated May 31, 1950. It consists of a horizontal tank with face plates at either end. Each face plate is marred with a thick rough weld line applied radially. The plates rotate in opposite directions causing the cellulosic materials, when mixed with water, to form a vortex in the center of the tank. The motion causes the substrate fibers to separate.

The mill was operated in the following manner. One hundred and ten pounds of water were added to the tank and the mill was put into operation-- while 4.4 pounds of whole newspaper were added. The temperature rise and electric power consumption were noted for each run with samples taken at various times during each run. Various additives such as sulfamic acid, corn oil, enzyme broth and steel nailpoints (10 mm long by 2.5 mm in diameter) were added at the start of each run in an effort to improve the efficiency of the mill. Hot water was added in place of the cold tap water in one run. The mill speed was increased from 647 rpm to 828 rpm in another run in an effort to increase the mill efficiency. Table 4 summarizes the data from the various runs.

This study shows the Retting mill capable of producing a product equal to approximately 80% of the product produced by ball milled newspaper at an energy level of approximately 1/2 k.w.h. per pound of dry newspaper. Chemical additives such as wetting agents, nailpoints, enzyme broth, corn oil, sulfamic acid or even the absence of printing ink had little effect on the efficiency of the Retting mill. Changes in physical conditions such as temperature of the water and speed of the mill had little effect on the efficiency of the mill.

ATTRITOR MILLING

Union Process Inc. of Akron, Ohio was contacted to process newspaper, both wet and dry, through an attritor mill.

Table 4. 24-hr Relative Yields for Retting Machine Treated Newspaper

Operating Conditions	24-hr Relative Yield for Various Processing Times			
	1 hr	2 hr	3 hr	4 hr
Newspaper with nat[illegible]nts	0.65	0.77	--	0.70
Newspaper with Tween 80 (20 g)	0.85	--	0.84	0.88
Unprinted newspaper	0.67	0.72	0.66	0.66
Newspaper in hot water (130°F)	0.62	0.70	0.59	0.59
Newspaper w/sulfamic acid (1/2 lb.)	0.51	0.62	0.60	0.60
Newspaper (agitation = 828 rpm)	0.68	0.75	0.91	0.73
Newspaper (agitation = 647 rpm)	0.83	0.83	0.84	0.89

An attritor is a vertical cyclinder containing small steel or stone balls. A steel shaft having steel extensions is rotated in the tank containing the balls. Grinding is obtained by the motion of the balls on the newspaper added to the mill. A no. 1-S pilot laboratory, model attritor was used. It has a 1 1/2 gallon grinding tank with 50 lbs. of 3/8" steel balls. A load of 400 grams of hammer milled newspaper was used for dry grinding and a 4% concentration of hammer milled newspaper in hot water was used for wet grinding (140 grams of hammer milled newspaper with 3,360 grams of hot water was used to make the 4% slurry).

Preliminary data indicates that processing hammer milled newspaper in an attritor mill might yield an excellent product for the enzymatic conversion of cellulose to glucose. However, this preliminary study lacks data regarding energy costs, process optimization and reproducibility. Much additional work using newspaper and other substrates processed in the attritor is necessary before any valid conclusions can be formulated.

TWO ROLL MILLING

Background

The roller crusher was probably first used, in the form of three rolls, to crush grain and beans and later to crush sugar cane as early as the 15th century. Rolls were first made to be used in a horizontal form in the eighteenth century and were generally driven by water wheels. There are many types of roll crushers. Their essential features are that they consist of

at least one roll rotating on its principal axis and ripping material particles between its surface and another surface to compress and break them. Roll crushers may consist of one, two, three, four or six or more cylinders, which are usually horizontal and revolve either towards each other or toward a plate. If they consist of more than one roll, the diameters and speeds of the rolls may differ. The distance between the rolls is usually adjustable. The action of the roll crusher is very similar to that of the jaw crusher. Instead of jaws moving in a horizontal direction relative to each other, the cylinder surfaces move in combined horizontal and vertical movements so that the material being crushed is continuously and steadily induced into the smaller part of the mouth by the circular movement of the cylinders and the friction between the material and the cylinder surfaces. Advantages of roll crushers are many.

(1) It will handle wet as well as dry material.

(2) The product can be controlled so that there is a high yield with a narrow size range.

(3) The power consumption of the two-roll crushers is low compared with that of many other machines such as jaw and gyratory crushers (49).

(4) Roll surfaces can be heated or cooled by the circulation of fluid within the interior of the rolls.

One of the basic pieces of equipment used in the rubber and plastics industries is the two-roll mill which is quite similar in design and construction to the two-roll crusher (50). It was for many years the only recognized mixing equipment for extremely viscous and elastic materials. A two-roll mill is used in the rubber industry to perform such operations as mixing, warm-up, sheeting, strip feeding, washing, refining and cracking. A two-roll mill is used in the plastics industry primarily to warm-up and blend granular resin particles and to form sheets for further processing.

A mill consists of two cast-iron tempered surface rolls, sometimes with other metals for additional surface protection, placed horizontally in bearings and held in place by mill housings. The rolls are set close together and are adjustable by mill screws. The mill rolls are driven by a bull gear powered through a reduction drive connected to a large motor. The other end of the rolls are connected by pinion gears. Figure 1 shows a typical differential speed two-roll mill. The rolls are usually cored, except that mills used in the plastics industry are frequently bored to provide for more accurate cooling and heating. The operation of a typical two-roll mill consists of placing crude rubber or premixed stocks between the rolls and as it is heated up the stock forms a band around the roll.

There are many types of mills used in the rubber and plastics industries such as mixing mills, strip mills, warm-up mills, sheeting mills, cracker mills, washer mills, refiner mills and other special mills each having specific roll speed ratios and roll surfaces unique to the type of processing performed on each. Three major factors must always be considered aside from the matter of established operational procedures. These factors involve the

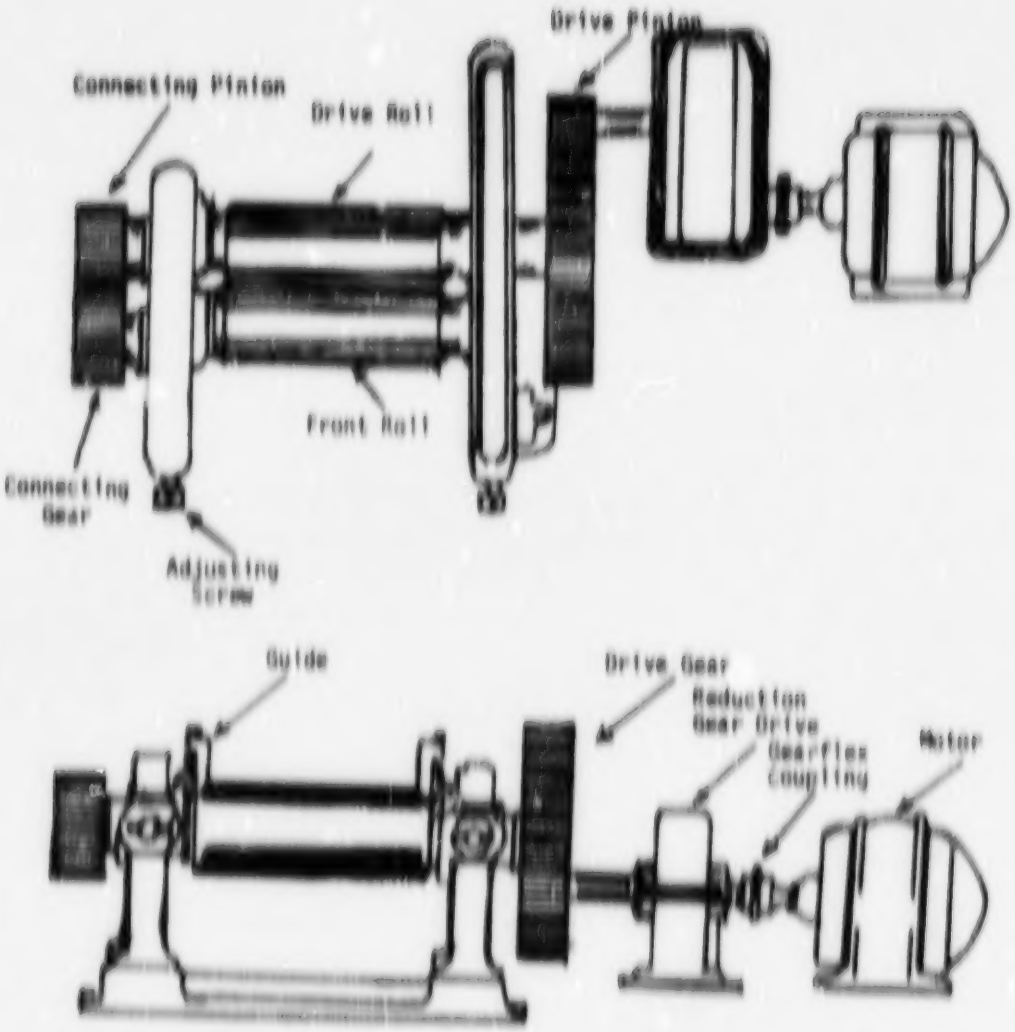


Figure 1. A Typical Differential Speed Two-Roll Mill

determination of the proper batch size and processing time, the need for consistent reproducibility and compound formulation.

There has been much work and many theories formulated about what happens to rubber when processed on a two-roll mill. It is generally agreed among researchers that during the first passage through the rolls, the rigid mass is stretched and ruptured, resulting in a tearing of the bonds of the micelles (51). Since rapid stretching is exothermic and rubber is a poor conductor, the temperature increases rapidly with the time of processing. Processing on a two-roll mill causes a ten-fold decrease from the initial molecular weight of natural rubber (52). The rate of degradation is greatest during the early stages and slows down eventually to almost zero, even though the shearing forces are still an appreciable fraction of those initially applied. Rubber molecules therefore suffer scission and breakdown until they reach a limiting molecular weight under normal masticating conditions, in the range of 70,000 to 100,000 for natural rubbers.

Many plastics, resins and fibers can be transformed into a rubberlike state by raising the temperature and adding plasticizers. Degradation under mechanical treatment has been noted for a great variety of natural and synthetic polymers.

Two-roll milling of cellulosic materials covered by this report follows none of the known procedures for the rubber and plastics industries but is a combination of several known methods. In preliminary work at Natick, only available laboratory equipment for rubber and plastics processing was used. Initial trials were made using various test substrates on a 3" x 8" two-roll mill made by Wm. R. Thropp & Sons Co., and a fixed roll speed ratio of 1.4 to 1 (23 rpm x 16 1/2 rpm). Next, a Farrell-Birmingham Co. 6" x 13" two-roll mill, and a fixed roll speed ratio of 1.4 to 1 (32 1/2 rpm x 23.2 rpm) was used. The only other available two-roll mill was a Farrell-Birmingham Co. 12" x 20" mill, with a variable speed drive. Each roll is driven by a D.C. motor which obtains its power from an A.C.-D.C. generator thus allowing wide variability in roll speed ratios. The mill rolls are flat rather than crowned, with very small roll clearances. The material is taken off the mill with a scraper after a specified processing time. The mill rolls can be heated or cooled as desired.

Experimental

Since the discovery midway through this program that two roll milling enhances cellulose susceptibility to enzymatic hydrolysis, significant progress has been made in translating favorable pilot scale milling results to full scale operation although much additional effort is needed. This preliminary data will be useful in determining future program direction.

Perhaps the most encouraging result so far is that two-roll milling has been shown to be effective using 3, 6 and 10-inch diameter two-roll mills on diverse cellulosic substrates such as newspaper, wood and cotton. This point will be clearly illustrated as the following data is presented concerning some significant milling parameters.

Roll Clearance--

The distance between the rolls of the mill (roll clearance) is very critical in determining enzymatic hydrolysis susceptibility of the product. In Figure 2 mean relative yield is plotted versus roll clearance for a hammer milled newspaper treated on the 10-inch mill for six minutes. Mean relative yield is the arithmetic mean of relative yields at the 4, 12 and 24 hour points on the hydrolysis curves. It is obvious that as the roll clearance decreases, susceptibility increases. This relationship would be expected to hold for other substrates besides newspaper and is probably a reflection of increasing pressure and tearing action as the rolls are brought closer together.

Processing Time--

Figure 3 shows the effect of processing time at various roll clearances on mean relative yield for a hammer milled newspaper on the 10-inch mill. For roll clearances of less than 0.040 inches, susceptibility increases with increasing processing time.

This behavior is similar to that noted for ball milling in that susceptibility improves with increasing processing time. However, whether it reaches a limiting value similar to ball milling (36) is unknown at this time. Additionally, the data indicates that processing times of 10 minutes or less can result in susceptibilities which could only be achieved by hours of ball milling.

Substrate Composition--

Figure 4 illustrates the substantial susceptibility improvements attained through two-roll milling (0.010 roll clearance) of maple and white pine wood chips. Three and twelve-fold improvements were noted for the white pine and maple respectively.

It should be pointed out that the presence of lignin is apparently affecting the results since the maplewood was milled for only 1 minute whereas the white pine had three minutes of milling and yet the maplewood was equally as reactive. This is not surprising in light of findings by other investigators using different pretreatments (see background review). In general, they observed that hardwoods were more readily susceptible to digestion by rumen fluids (enzymatic system) than softwoods.

Absorbent Cotton processed on the three and six-inch mills has exhibited twelve-fold increases in susceptibility over unmilled cotton. Figure 5 shows data for the six-inch mill. The authors believe that these impressive results are indicative of crystallinity changes and molecular chain scission processes occurring as the cotton is milled. Similar changes may have been observed by other researchers following ball milling of alpha cellulose pulp (background review). Alteration of lignin structure certainly cannot be responsible for the increased reactivity since there is no lignin in cotton.

Power Data--

Electrical power data was collected on the three-inch laboratory mill using newspaper. A cumulative watt meter was connected between the line

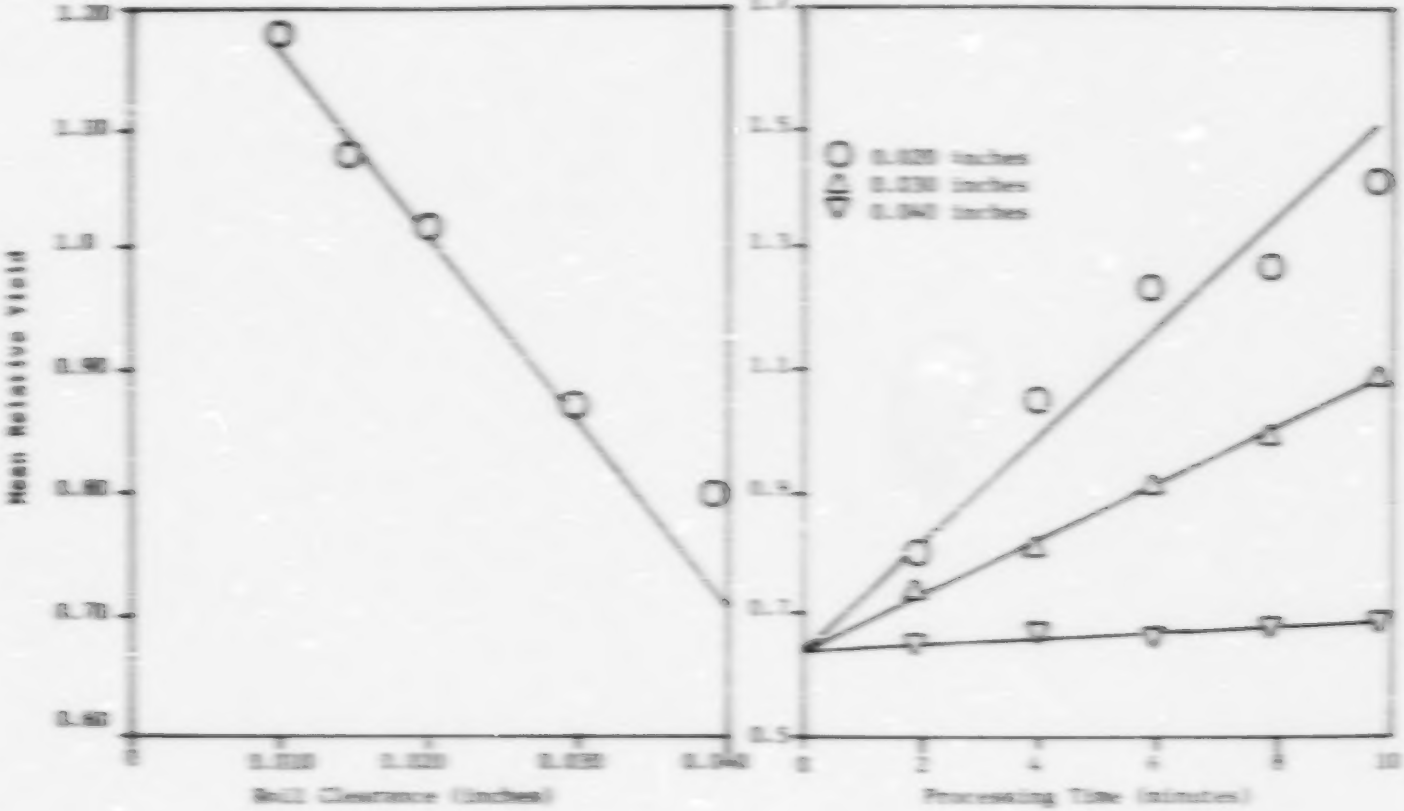


Figure 2. Effect of Roll Clearance on Mean Relative Yield of Two Roll Milled Newspaper. Roll Speeds: 25 fpm and 35 fpm; Rolls chilled; 1.25 lb. of newspaper; Roll diameter: 10 inches.

Figure 3. Effect of Processing time on Mean Relative Yield of Two Roll Milled Newspaper. Roll Speeds: 25 fpm and 35 fpm; rolls preheated to 130°F; 1.25 lb. newspaper; 0.020 inches roll clearance; Roll diameters = 10 inches.

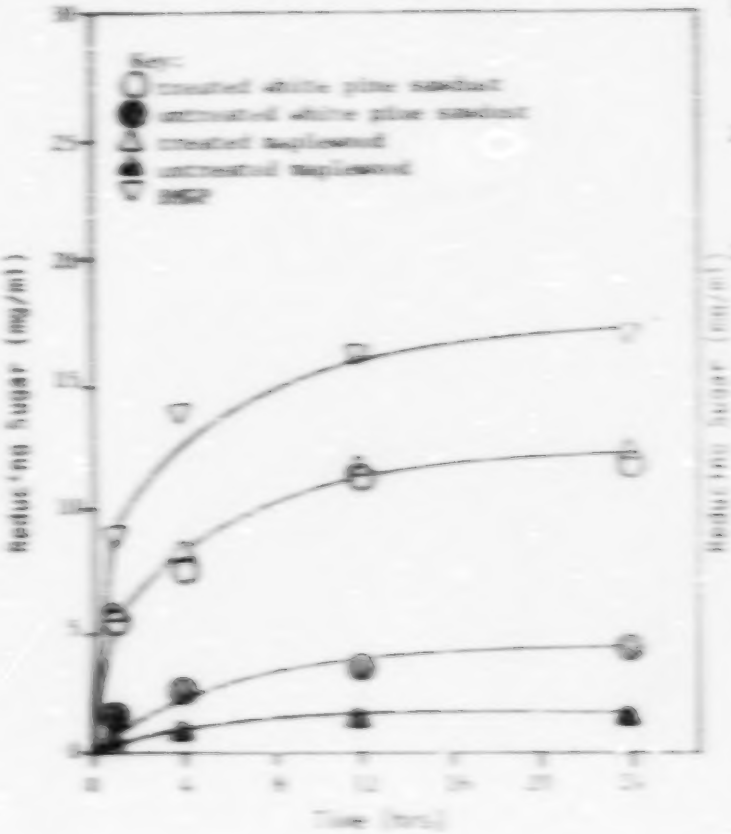


Figure 4. Effect of Two Roll Milling on Enzymatic Hydrolysis of White Pine and Hardwood Sawdusts. Roll diameters: 10 inches; Roll speeds: 35 and 55 fpm; Sample weight: 1.25 lb.; Processing times: 1 min for hardwood, 3 min for pinewood; Roll clearance: 0.002 inches.

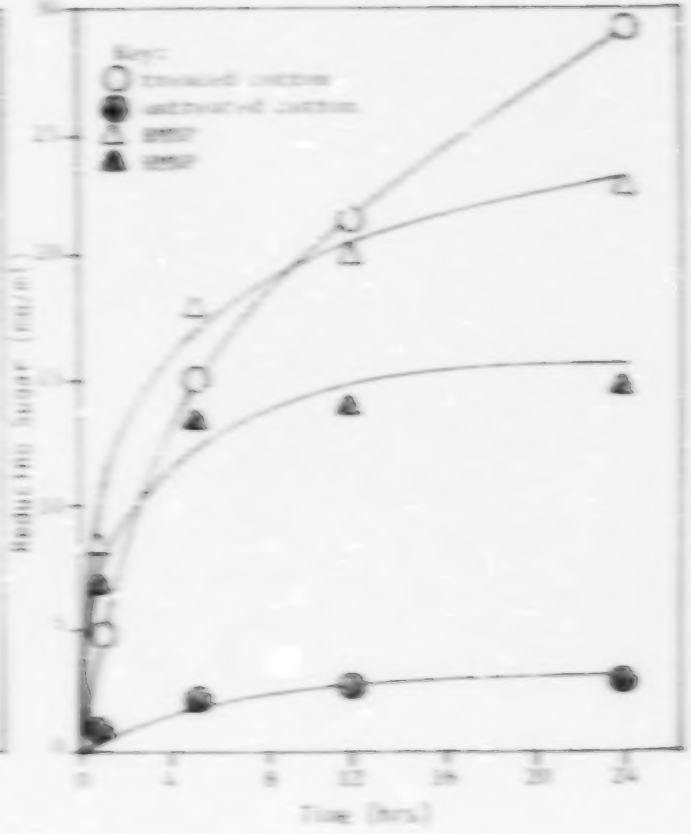


Figure 5. Effect of Two Roll Milling on Enzymatic Hydrolysis of Cotton. Roll diameters: 6 inches; Roll speeds: 36 and 11 fpm; Sample weight: 50 g; Roll clearance: 0.005 inches; Processing time: 2 min.

and the A.C. motor which operates the rolls.

From the very limited power consumption data of Table 5, it appears that as roll clearance is decreased, power consumption and substrate reactivity increases. For a product of comparable reactivity, power required per pound is at least 20% less than that for commercially ball milled newspaper as estimated by Nystrom (53). In addition, it is expected from conversations with equipment manufacturers, that this power figure should decrease as larger production scale two-roll mills are evaluated.

Table 5. Power Consumption for Laboratory Two-Roll Milling of Newspaper

Roll Clearance (inches)	Processing Time (minutes)	Sample Weight (g)	Mean Relative Yield	Power (kwh)	Power (kwh/lb.)
0.008	2	30	1.05	0.0576	0.872
0.010	2	30	0.93	0.0461	0.697

Sedimentation Volume--

Five gram samples of commercially ball milled and two-roll milled newspaper (six-inch mill at 0.010 inch roll clearance) were dispersed thoroughly in distilled water to give a total volume of 100 ml. The slurry was then shaken for 10 minutes, total volume measured and water added to bring it back to 100 ml. It was shaken for another fifteen minutes, volume was measured in a graduated cylinder and found unchanged. The mixture was allowed to settle in the cylinder and the volume occupied by the solids measured at various times. The results are presented in Table 6.

Table 6. Sedimentation Volume of Two-Roll Milled and Ball Milled Newspaper

Substrate	Sedimentation Volume (ml) 1 hr.	17 hrs.	144 hrs.
Ball Milled Newspaper	38.5	39.5	39.5
Two-Roll Milled Newspaper	31.0	31.5	31.5

The data clearly shows that the wet density of two-roll milled newspaper is greater than ball milled newspaper. This is important to the cellulose conversion process, since it implies that slurries of greater concentration will be possible in production scale hydrolysis vessels

thereby reducing reactor volume and hence capital costs over substrates which can only be slurried at lower concentration levels.

ANHYDROUS LIQUID AMMONIA

To study the disruptive effects of liquid ammonia on the lattice structure and chemical bonding of cellulosic materials, experiments were begun on a number of substrates. These include softwood and hardwood sawdusts, newspaper and a lignin-free cellulose, Avicel PH102 (American Viscose). In some cases ammonia treatment was performed following two-roll milling of the material.

Liquid ammonia experiments were carried out in two ways: 1) in an open Dewar flask at atmospheric pressure and a temperature of -55°C, 2) in a Parr Instrument Co. Pressure Reactor, Model No. 4562 at temperatures and pressures of 25°C, 10 atmospheres and 60°C, 24 atmospheres.

The anhydrous liquid ammonia used was 99.99% purity (Matheson Gas Products Co.). For the open Dewar experiments, ammonia gas was condensed by passing over a stainless steel coil cooled to -60°C by means of a Model C-60 Cryocool Unit (Neslab Instruments Inc.). When using the pressure reactor, the gaseous ammonia was first condensed into a 300 ml flask immersed in a dry ice-alcohol bath at -70°C and then delivered through a tube into the precooled reactor by applying a pressure of dry nitrogen to the surface of the liquid ammonia. The reactor was then sealed and allowed to come to room temperature or heated in a hot water bath. Volume of the reactor was 450 ml and sufficient liquid ammonia was used (usually 150 ml) to insure that the liquid phase was still present at the higher temperatures. In the experiments thus far atmospheric moisture and oxygen have not been rigorously excluded.

Materials to be treated were usually oven dried overnight at 103°C and 10 gm samples were taken for any particular run. At the conclusion of a run, the reactor was vented and the remaining ammonia allowed to evaporate at room temperature overnight or until the odor of the gas was no longer detectable.

Effect of the ammonia treatment was assessed by enzymatic hydrolysis employing the cellulolytic enzyme preparation derived from a mutant of the fungus Trichoderma viride (54). After evaporation of the ammonia, the 10 gm samples were divided in two and hydrolyzed as 5% slurries by the procedure given in Appendix B. The concentration of reducing sugars obtained was determined by the spectrophotometric dinitrosalicylic (DNS) acid method (55) and in some instances also by liquid chromatography (56). The DNS method determines the total of reducing sugars while the chromatographic method determines only the xylose, glucose and cellobiose.

Avicel, PH102, a commercial microcrystalline cellulose obtained from the dilute acid hydrolysis of cotton, was taken as representative of a lignin-free cellulose. It was held in liquid ammonia for 3 hours at 60°C, 24

atmospheres pressure. Enzymatic hydrolysis of this material gave the results presented in Figure 6. Data for ball milled newspaper (BMNP), hydrolyzed at the same time, are also given for comparison. It is interesting that the initial rate of hydrolysis of the ammonia treated Avicel is intermediate between the untreated control and the BMNP, which is generally considered to be more nearly amorphous because of the ball milling. This could be indicative of a disordering of the highly crystalline Avicel by the action of the ammonia.

Figures 7, 8 and 9 present hydrolysis data for two hardwood samples, yellow birch and maple and the softwood white pine.

Figure 7 was obtained with yellow birch sawdust that passed a 35 mesh sieve. It shows an increasing effect of ammonia pretreatment with increasing severity of experimental conditions. The points at 26 hours are the sums of xylose, glucose and cellobiose values determined by liquid chromatography. The hydrolysis curve for 60°C, 24 atmospheres closely paralleled that of ball milled newspaper. In an experiment to determine the importance of the length of time of treatment, 1/2 hour of contact with liquid ammonia gave approximately the same result after 24 hours of hydrolysis as did 3 hours of contact at 60°C and 24 atmospheres. The chromatographic data indicated that at the higher temperature and pressure relatively more glucose was produced at the expense of xylose and cellobiose than under room temperature conditions.

In Figure 8 data are presented for a sample of maplewood that had received only one minute processing time on a 10-inch diameter two-roll mill. A two-fold increase in production of reducing sugar is shown for the ammonia pretreated sample. Again it appears that 1/2 hour of treatment with liquid ammonia is as effective as 3 hours. The 24-hour hydrolysis value for BMNP was midway between the maplewood values. Satisfactory agreement between the 24-hour DNS values and liquid chromatography values was also found in this instance.

Experiments with white pine sawdust, 35 mesh, demonstrate the often observed variability in the behavior of hardwoods and softwoods towards particular chemical and/or physical treatments. In this instance, improvement in enzymatic hydrolyzability was approximately three-fold less than in the case of the hardwood birch sawdust, 35 mesh, Figure 9. Also, treatments at room temperature, 10 atmospheres and 60°C, 24 atmospheres and for 3 hours or 24 hours under the room temperature conditions gave little or no difference in the hydrolysis results. Presumably, an explanation for the differences in hard and softwood behavior lies in the higher lignin content of the pinewood as well as differences in wood structures which can limit accessibility.

Included in Figure 9 are data for white pine shavings that had been ground in the two-roll mill prior to treatment with liquid ammonia at 60°C, 24 atmospheres. The milling alone provided a somewhat higher value than that from ammonia pretreatment alone. Treating of the milled material with ammonia increased the reducing sugar yield by approximately 10%.

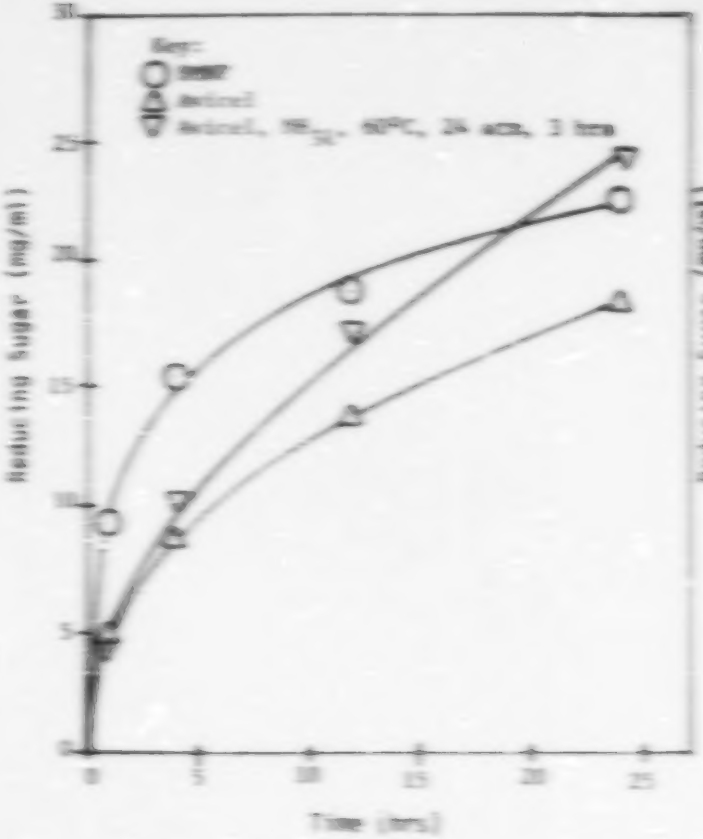


Figure 6. Effect of Liquid Ammonia Pretreatment on the Hydrolysis of Avicel.

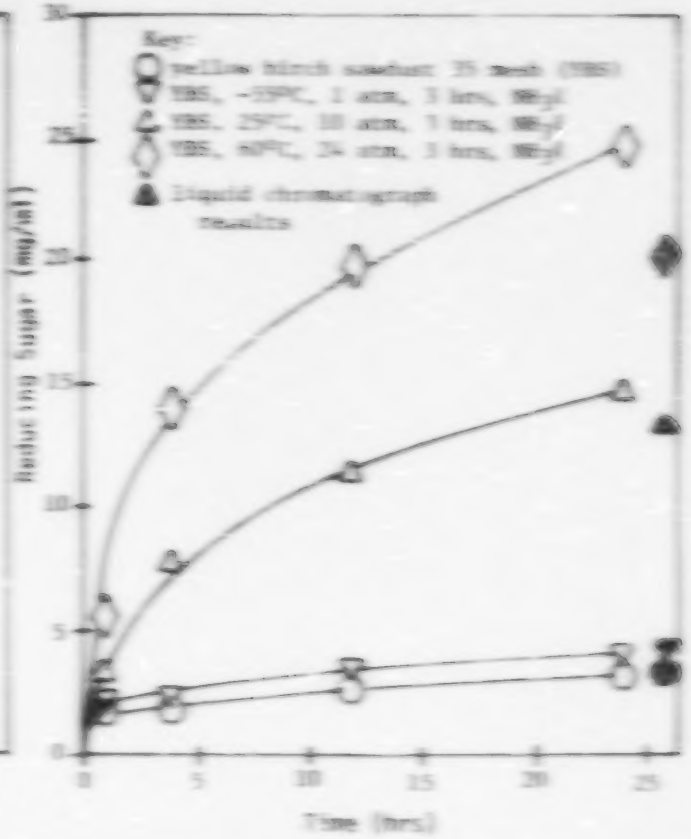
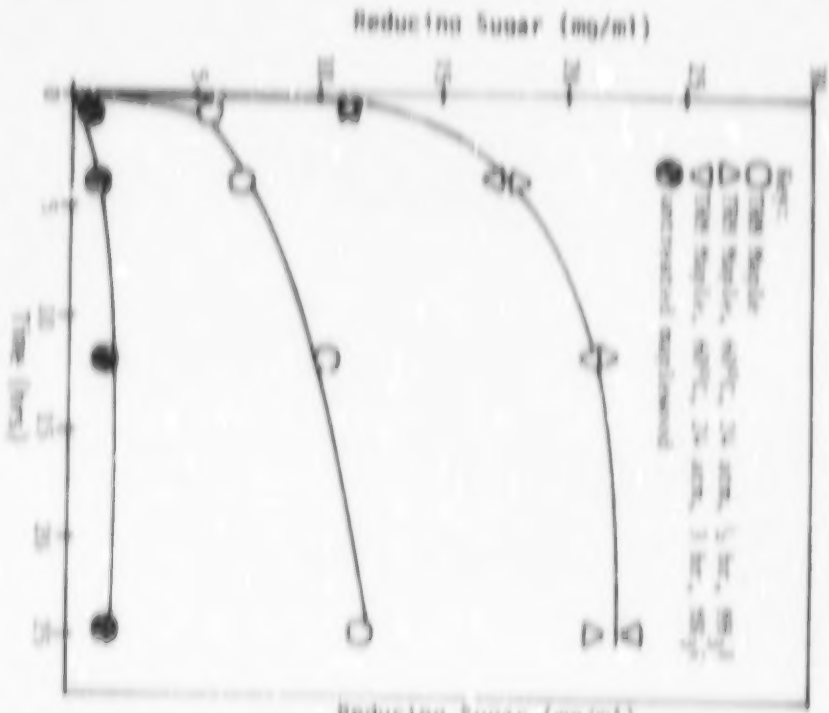
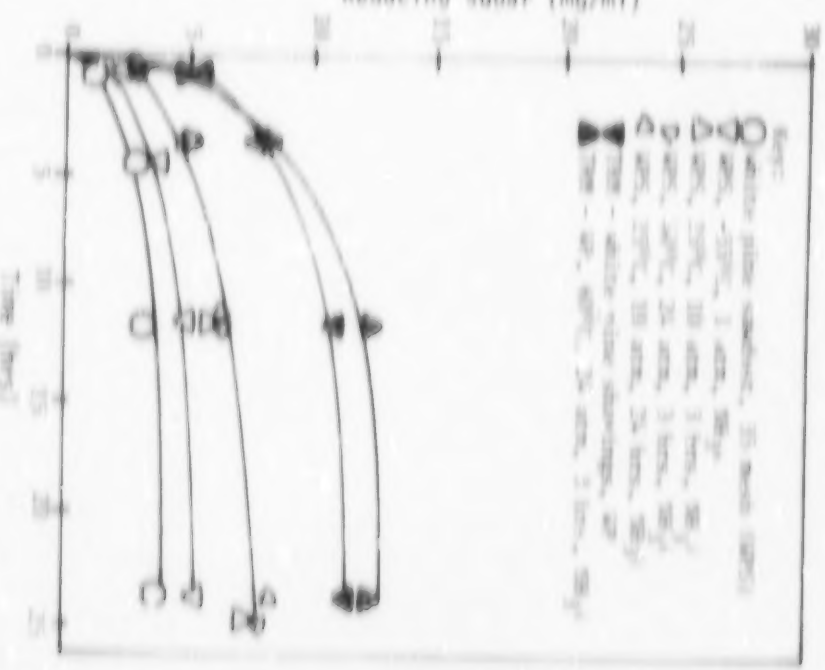


Figure 7. Effect of Liquid Ammonia Pretreatment, Under Varying Conditions of Temperature, Pressure and Time, on the Hydrolysis of Yellow Birch Sawdust, 35 mesh.

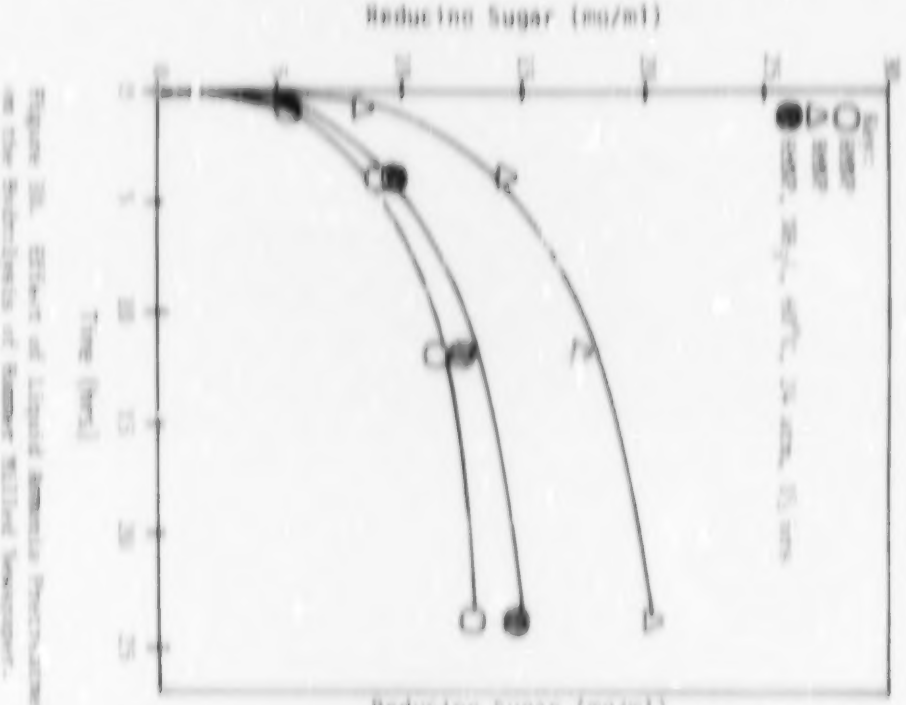

Reducing Sugar (mg/ml)

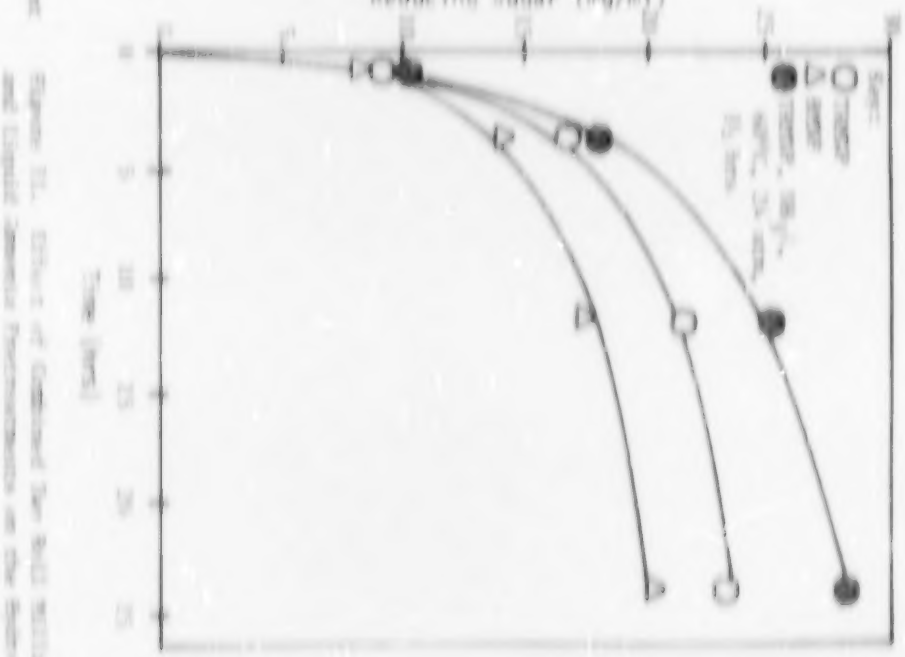

Reducing Sugar (mg/ml)

Figures 10 and 11 present data from initial experiments on the pretreatment of newspaper samples.

In Figure 10, a 12% increase in hydrolysis yield over the control is seen for the shredlike hammer milled newspaper after a 1 1/2 hour, 60°C, 24 atmospheres ammonia pretreatment. The results for the non-treated, very finely powdered ball milled newspaper are shown for comparison.

A combination of two-roll milling and liquid ammonia pretreatment appears to have a significant effect as seen in Figure 11. Initially, hammer milled newspaper was ground in the two-roll mill. The additional ammonia treatment appears to effect a 22% increase in yield of reducing sugar after a 24-hour hydrolysis. Results for a commercially ball milled newspaper, no further pretreatment, are shown for comparison.


Reducing Sugar (mg/ml)


Reducing Sugar (mg/ml)

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APPENDIX A

DELIGNIFICATION PROCESSES

PULP AND PAPER INDUSTRY

In the pulp and paper industry, the major objective in chemically treating wood is to produce a long fibered paper product of high strength and varying degrees of whiteness depending upon end use. To accomplish these aims, chemical processes such as bleaching and delignification are used. Excellent reviews of the numerous bleaching and delignification processes are provided by Jahn (57), McGregor (58), Rydholm (59) and Tomlinson (60). A brief summary follows:

Sulfite

In this delignification process, wood chips are cooked at 130-150°C in pressurized vessels containing water, sulfur dioxide, and bisulfites of various bases. Lignin combines with the sulfur dioxide and/or bisulfite and in so doing is solubilized. Some hemicelluloses are hydrolyzed and a portion of the wood cellulose degraded. Typical pulp yields are 40-50% of the weight of the wood charged to the digester. Numerous by-products are formed during the sulfite cooking process including fermentable sugars, carbon dioxide, acetic and formic acids, methanol, and cymene.

Soda

Wood chips are cooked in pressurized vessels containing aqueous solutions of 17 to 25 percent sodium hydroxide on a dry wood weight basis for periods ranging from 2 to 6 hours and at temperatures of 165 to 175°C. Typical yields are from 43 to 48 percent of original weight. A significant portion of the hemicelluloses are solubilized and lignin is removed. The waste liquor contains cooking chemicals, fermentable sugars and lignin fragments but is missing the troublesome secondary reaction by-products found in the sulfite process.

Kraft

In the kraft or sulfate process, the same operating conditions as the soda process are utilized. The only difference is that a mixture of sodium hydroxide and sodium sulfide is charged to the reaction vessel. As a result, higher strength pulp in greater yield with the same degree of delignification is obtained. This process and the sulfite process are the two most widely used commercial chemical pulping processes.

Semi-chemical

This involves a two-stage production process. The first is a mild chemical action which loosens and partly removes lignin; the second step completes the separation of fibers by mechanical means. The first stage quite commonly involves the use of the neutral sulfite process - sodium sulfite buffered with sodium carbonate or sodium bicarbonate to maintain neutral pH. Modified kraft or sulfite processes are also used. The second stage involves the use of mechanical equipment such as refiners or rod mills.

With all semi-chemical processes, high yields (60-70%) can be expected as less hemicellulose and cellulose is attacked than in chemical pulping processes. On the other hand, less lignin is removed.

Bleaching

The importance of bleaching in pulp manufacture is to impart whiteness or brightness to the final product. This can be done in two ways - lignin bleaching or lignin removal. In lignin bleaching, chromophores of lignin and other impurities are destroyed without removal of substance. If greater color stability and higher brightness is desired, multistage processing including an alkali extraction stage is used to oxidize and remove the lignin with minor cellulose loss. Hence this type of bleaching represents a continuation of the cooking processes using much more selective and at the same time expensive chemicals.

Among the most commonly used bleaching agents are elemental chlorine, chlorine dioxide, hypochlorites, and chlorites. Others include permanganates, and peroxides with reducing agents such as zinc hydrosulphite used extensively for the reduction of color in groundwood pulps.

Other

Aqueous solutions of nitric acid (2-8%) have been found effective in delignifying agricultural residues (rice straw, bagasse, etc.), but have produced low yields (37-44%) due to partial removal of cellulose and hemicellulose resulting from hydrolysis. The process involved oxidation and nitration of the lignin into fragments which are removed by an alkali extraction step.

Through the use of ethyl alcohol in various proportions with aqueous nitric acid, it is possible to increase yield to 40-50% due mainly to the inhibitory action of the alcohol on carbohydrate hydrolysis by nitric acid (61).

Favorable aspects of alcoholic nitric acid pulping of bagasse include (a) low pulping temperatures (90°C), (b) atmospheric pressure, (c) short pulping time (2-4 hours), (d) low acid concentration, and relatively good yields.

Aronovsky and Gortner (62) looked at aqueous solutions of aliphatic monohydroxy and polyhydroxy alcohols and found that n-butyl alcohol, n-amyl alcohol, isoamyl alcohol and ethylene glycol with an acid or aluminum chloride catalyst will delignify wood chips. Using aspen, a cooking time of 1.5 to 3.0 hours at 180°C, and a 1:1 solution of butyl alcohol in water, yields of 48-55% were obtained with a lignin content to 6-10%.

The steps involved in delignification using alcohols are believed to be alcoholysis causing partial depolymerization followed by dissolution. If the solvent power is poor, only the lower molecular weight lignin fragments will dissolve; the remainder to undergo condensation reactions and repolymerization. Schuerch (63) has shown that the ability of solvents to dissolve or swell isolated lignins increases as the hydrogen bonding capacities of the solvents increase and as their solubility parameters approach a value of eleven.

Sulfolane (tetramethylene sulfone), a highly polar solvent with substantial chemical and thermal stability, has been used in aqueous solutions to delignify aspen wood chips. Using a 30 vol. % solution of sulfolane in water, Clermont (64) was able to attain rapid delignification. He obtained a 67% yield with a 6.4% residual lignin content after cooking for 1 hour at 175°C under pressure.

Yields were dependent on the amount of water in the cooking liquor and the initial pH of same. With increasing amounts of water, more lignin and also more carbohydrate was removed.

APPENDIX B

PRETREATMENT EVALUATION PROCEDURES

The evaluation of any particular pretreatment is carried out in two phases, 1) the enzymatic hydrolysis of the substrate under investigation and 2) the analysis of the hydrolysate for reducing sugars by the dinitrosalicylic (DNS) acid method (55).

The enzymatic hydrolysis is carried out on 5% slurries (dry weight basis) of substrates in the presence of enzyme preparations of *T. viride*, adjusted to constant enzyme activity, usually 1.0 filter paper units/ml. Sodium citrate buffer (0.05 m) is used to control pH to approximately 4.8 and small amounts of merthiolate (.01%) are added as preservative. Hydrolyses are carried out in a vibrating incubator at 50°C with small aliquots removed for sugar analysis at 1, 4, 12 and 24 hours.

The DNS analyses for reducing sugar are carried out spectrophotometrically at 550 nanometers. Measured values of transmittance are converted to concentration values by reference to a standard curve obtained from solutions of known glucose content.